

The Effects of 2, 4-Dichlorophenoxyacetic Acid, Pentachlorophenol and Mixtures of These on an Aero-aquatic Fungus.

Peter David Premdas* and Bryce Kendrick

Department of Biology

University of Waterloo

Waterloo, Ontario N2L 3G1, Canada

ABSTRACT

The biocides pentachlorophenol (PCP) and 2,4-dichlorophenoxyacetic acid (2,4-D) are applied in oil or oil-based carrier formulations. In lentic systems these accumulate in the surface microlayer, the top 60-150 μm of the water. We have designed a unique bioassay system involving the germinative buoyant propagules of an aero-aquatic fungus, *Pseudoaegerita matsushimii*, which can assess the toxicity of contaminants present in this zone.

PCP dissolved in the oil emulsion carrier and oil carrier alone inhibited propagule germination. Mixtures of 1,2 and 3 parts 2,4-D to 1 part PCP stimulated germination at low concentrations but inhibited germination at high concentrations. Mixtures of 2 and 3 parts PCP to 1 part 2,4-D inhibited germination at all concentrations tested. Pesticide mixtures differed from their components with respect to net toxicity.

INTRODUCTION

2,4-DICHLOROPHENOXYACETIC ACID. 2,4-Dichlorophenoxyacetic acid is most widely used broad-leaf terrestrial herbicide in North-America. It is used for the control of rooted aquatic macrophytes and floating aquatic plants (Rice and Switzer 1989).

During a 1974 sampling of eight Ontario watersheds, 2,4-D was found in 39% of all water samples. In addition, 60% of farm ponds and wells were found to be contaminated by phenoxy herbicides, 32% of these by 2,4-D (Frank et al. 1978). This chemical has been detected in aquatic ecosystems three weeks to four months following application (Frank and Comes 1967, Wojtalik et al. 1974, Shultz and Harmon 1974.) and it may persist even longer in Canadian aquatic systems because of the prevailing cool temperatures.

2,4-D is extensively used as a pre-logging or harvest defoliant and is applied by aerial spraying. Drift ratios from the target site of up to 60% are common and, apparently, commercially acceptable. Total lake water levels of 1.0-3.0 mg/l attributed to drift are not uncommon (NRCC 16075, 1978). To date, no studies of its biological effects have been conducted at the surface microlayer where initial accumulation of 2,4-D and 2,4-D esters dissolved in oil-based carriers, must occur.

PENTACHLOROPHENOL. Pentachlorophenol (PCP) is one of the most widely used pesticides in Canada. Introduced in 1936 as a wood preservative it has seen widespread use as a fungicide, pre-harvest defoliant, algicide, molluscicide, paint additive and insecticide (Richardson 1978). Its widespread applicability stems from its action as a potent uncoupler of oxidative phosphorylation (Weinbach 1956).

PCP in the environment may be derived from many sources. Konase

* Present address: Department of Biology, Queen's University, Kingston, Ont. K7L 3N6

and Henning (1988) identified as many as 22 potential PCP release points standard wood treatment facilities alone. PCP may reach aquatic systems several ways; surface runoff and sludge disposal are certainly prime contributors. Significant amounts of PCPs are released from PCP-manufacturing and use plants every year due to the poor quality of stack emission-filtering systems (1975) and may be deposited on aquatic systems. Drift from aerial spraying is also important as the final destination may often be kilometers away from the initial release site. PCP used as a molluscicide or pesticide is usually deliberately introduced into the aquatic system as a control agent. On-site preservation commonly used at sawmills and logging camps, may contaminate the aquatic environment due to run-off and unregulated disposal into ponds and lakes.

THE SURFACE MICROLAYER. In lentic systems, there is a thin surface layer which often contains pesticides at levels more than one hundred thousand times those sampled in the remainder of the water column (Sodergren 1979). This zone, or surface microlayer, may be between 60 - 150 μm thick (Maguire et al. 1982, Duce et al. 1972) and is composed of naturally occurring and anthropogenically-derived lipophilic substances which concentrate lipid-soluble chemicals. As both PCP and 2,4-D are almost universally dispersed in oil carriers, concomitant contamination by these two chemicals at the air-water interface is a very real possibility, especially in areas supporting heavy logging and forestry-related industries. The environmental impact of biocides in the microlayer has rarely been addressed because none of the standard bioassay systems can be localized with sufficient precision to operate specifically at the air-water interface.

In heterotrophic aquatic systems which depend mainly on fallen leaves to meet their energy needs, the primary food converters are aquatic dikaryotic fungi and anamorphs (Baerlocher and Kendrick 1974). In small lentic systems e.g., woodland ponds, aero-aquatic fungi predominate. Like other aquatic fungi, these colonize submerged detritus e.g., leaves or twigs. However, their propagules are produced above the water surface, in air. The propagules are produced because they entrap air during development (Michaelides 1982, Michaelides and Kendrick 1982). After maturity they become detached and float about on the water surface where they come into contact with and colonize autotrophic leaves as these fall into the pond. We have developed a bioassay for pesticides present in the surface microlayer using propagules of the aquatic fungus Pseudoaegerita matsushimae.

MATERIALS AND METHODS

THE BIOASSAY SYSTEM. Propagules were obtained from partially submerged decaying maple leaves according to the method outlined by Michaelides (1979). These propagules were manually transferred on 9 mm diameter nitrocellulose filter disks, approximately 50 propagules per disk. Disks were placed in microcentrifuge tubes, and the test substance was added in 20 μl aliquots. This quantity was sufficient to completely cover a disk without dislodging any propagules.

The tubes were closed and placed in an incubator for 16 hours at 25°C. Subsequently, filters were mounted on slides, stained using Lactophenol blue and heated to kill the propagules. Preparations were then examined microscopically to assess the number of propagules which had germinated. The presence of germ tubes was used as the criterion for establishing germination. Bioassays were carried out a total of five times at each formulation concentration tested. A more detailed methodology may be obtained from the primary author.

Analytical grade 2,4-D (SigmaTM) was dissolved in an emulsifiable concentrate carrier [EOC] - (98 parts Pharmaceutical-grade light mineral oil [FisherTM Trading CompanyTM] to 2 parts Tween-80 [FisherTM]). Stock solutions of 100 g were made up as follows: 100 mg 2,4-D were added to 1.0 ml carrier in a foil-wrapped 1 dram vial. This was placed on a Vortex mixer (FisherTM) at high speed for a minimum of 5 minutes, to completely dissolve the 2,4-D. Subsequent concentrations from 10.0 g/l to 0.1 mg/l were made by serial dilution of the stock. The following final concentrations were obtained: Control (distilled water), carrier only, 100 g/l, 10.0 g/l, 1.0 g/l, 100 mg/l, 10.0 mg/l, 1.0 mg/l and 0.1 mg/l solutions were stored in the dark at 10°C between experiments to ensure chemical stability.

Bioassays were also carried out using 2,4-D-PCP mixtures at the following ratios: 1 part 2,4-D to 1 part PCP, 2 parts 2,4-D to 1 part PCP, 3 parts 2,4-D to 1 part PCP, 2 parts PCP to 1 part 2,4-D, 3 parts PCP to 1 part 2,4-D. Stock solutions were made as follows: 100 mg 2,4-D were added to 1.0 ml EOC in a foil-wrapped vial, and the mixture was placed on a Vortex mixer at high speed for a minimum of 5 minutes. Analytical grade PCP, (SigmaTM), was added to produce the appropriate ratio, and the mixture was stirred continuously overnight in the dark to ensure complete solvation. The mixture was subsequently stored in the dark at 10°C to prevent photolysis of the PCP component and to ensure chemical stability of the mixture as a whole.

STATISTICAL TREATMENT OF DATA. Data from 2,4-D and 1, 2 and 3 parts 2,4-D to 1 part PCP were graphed using interpolated CricketTM and StatworksTM programs. Results were described into a second order polynomial. This method was used as 2,4-D and several 2,4-D mixtures initially stimulated, then depressed propagule germination. Maximal stimulation of germination was taken as the point where the slope of the graph was effectively zero. In order to determine IC50s (the toxicant concentration that inhibited germination in 50% of the individuals), data from the maximal stimulation point to the point of total germination inhibition were subjected to probit analysis to yield a 50.0% inhibition (IC50). Data from treatments using PCP and 2 and 3 parts PCP to 1 part 2,4-D which did not stimulate germination were analyzed using the probit method.

RESULTS AND DISCUSSION

ANOVA of 2,4-D bioassay values revealed that 2,4-D in an EOC carrier stimulated germination rates from 60.0% to approximately 97.0% over concentrations from 0.1 mg/l to 3.0 mg/l (Figure 1). As the levels of 2,4-D were increased from 3.0 mg/l to 100 g/l, germination values decreased from 97.0% to 60.0%. The carrier alone depressed germination to approximately 60.0% but 2,4-D and 1,2 and 3 parts 2,4-D mixtures dissolved in the same carrier had a stimulatory effect.

PCP alone did not stimulate germination and yielded an IC50 at 0.017 g/l (Table 1). A 1:1 mixture of 2,4-D and PCP induced maximal stimulation of germination at 2.0 g/l; however, this value decreased as the ratio of 2,4-D to PCP was increased from 1, 2 and 3 parts. Unassisted 2,4-D yielded an IC50 at ~ 7.5 g/l. A 1:1 mixture of 2,4-D and PCP produced an IC50 of approximately 7.5 g/l; this value decreased to 2.8 g/l as ratios were raised to 3 parts 2,4-D. In all, 2,4-D and mixtures containing 1,2 and 3 parts 2,4-D all stimulated germination at concentrations then inhibited germinations as concentrations were increased (Figures 1 and 2). Mixtures of 2 and 3 parts PCP to 1 part 2,4-D did not stimulate germination and yielded IC50s of 0.019 and 0.017 g/l respectively (Table 1). A sample bioassay using a 2:1 mixture of PCP and 2,4-D is shown in Figure 3.

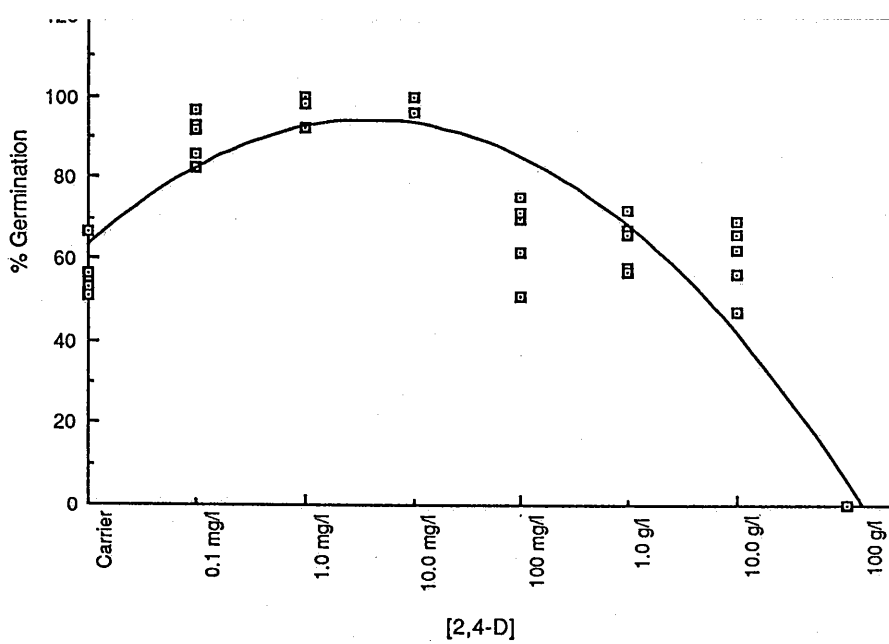


FIGURE 1. % Propagule Germination vs. 2,4-D Concentration.
 Each point represents a separate experiment involving 50 propa
 $y = 62.884 + 23.809x - 4.5517x^2$ $R^2 = 0.842$

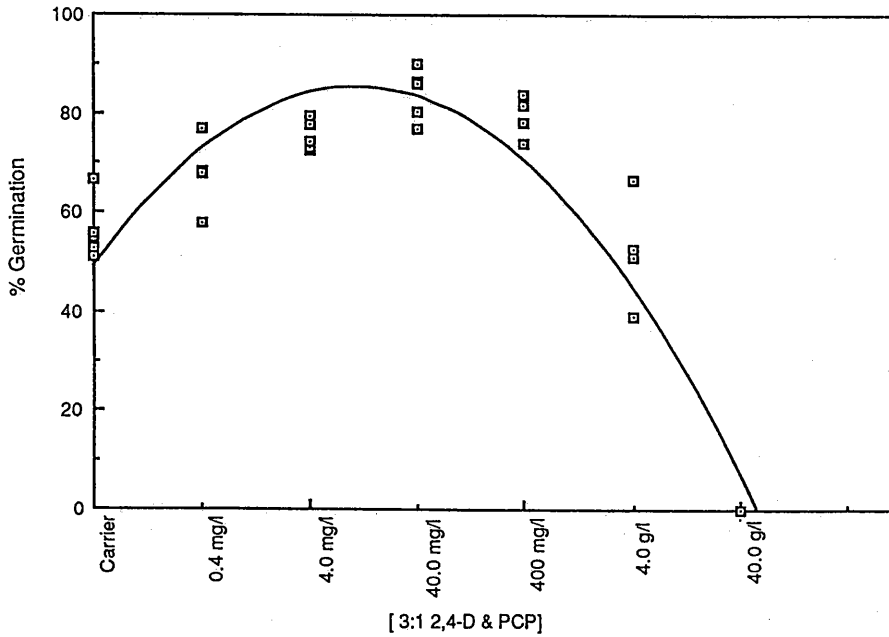


FIGURE 2. % Propagule Germination vs. 3:1 2,4-D & PCP Concer
 Each point represents a separate experiment involving 50 propa
 $y = 48.877 + 30.020x - 6.1526x^2$ $R^2 = 0.890$

levels of toxicity and did not stimulate germination. With the exception of the 3 mixture of 2,4-D and PCP which stimulated germination to ~ 94.0%. Unassis 2,4-D and 1 and 2 parts 2,4-D to 1 part PCP all stimulated germination from ~ 60.0% (carrier only) to levels not significantly different from those of control va (distilled water), ~ 97.0% (Figure 4). Toxicities (IC50s) also increased in 2,4-D PCP mixtures as ratios of 2,4-D were increased. As expected, PCP and mixtu containing higher ratios of PCP, a respiratory inhibitor, were more toxic to *P. matsushimae*.

TABLE 1

IC50S AND MAXIMAL STIMULATORY CONCENTRATION OF 2,4-D, PCP AND 2,4-PCP MIXTURES.

Toxicant mixture	Maximal stimulatory concentration [g/L]	IC50 [g/L]
PCP	none	0.016
2,4-D	0.003	7.940
1 2,4-D/1 PCP	2.000	7.516
2 2,4-D/1 PCP	0.015	3.000
3 2,4-D/1 PCP	0.008	2.830
2 PCP/ 1 2,4-D	none	0.019
3 PCP/ 1 2,4-D	none	0.017

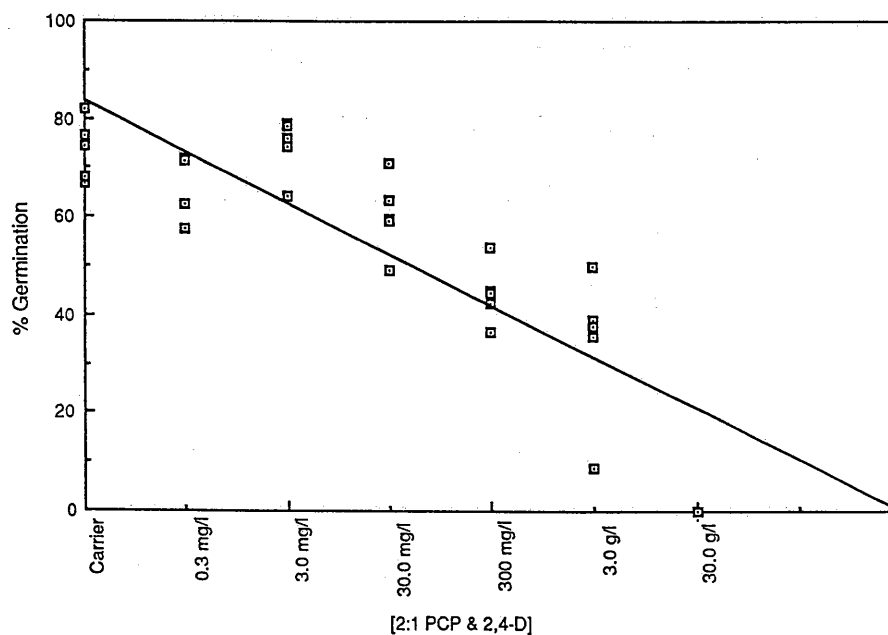


FIGURE 3. % Propagule Germination vs. 2:1 PCP & 2,4-D Concentration. Each point represents a separate experiment involving 50 propagules. $y = 5.4257 - 0.42579x$ $R^2 = 0.746$ Probit Graph

Several authors have reported increased fungal activity (growth and reproduction) following exposure to 2,4-D (Balasubramanian and Rangasv 1973 , Katan and Eshel 1973 , Yoder and Klos 1982). Pseudoaegerita matsushimae was, in several cases, affected by equivalent or smaller amount of 2,4-D than plant pathogens or soil fungi reported in the literature. Gaur and I (1972) tested growth rates of seven Rhizobium species in media containing 2000 mg/l 2,4-D. Growth of two species was slightly suppressed at concentrations > 50 mg/l, two were suppressed only at concentrations > 250 mg/l, while one was stimulated at concentrations >250 mg/l. The population of some soil fungi are depressed by 2,4-D while others are stimulated (Ilyin 1972). Microbial nitrate mineralization in fields sprayed with phenoxy herbicides was reported to be 3-4 times that of untreated controls (Grassbard 1971).

The incidence of some crop diseases caused by pathogenic fungi was increased following 2,4-D application (Katan and Eshel 1973). Low (sub-letal) concentrations of 2,4-D (Mecoprop™) were reported to increase the incidence of Gaeumannomyces graminis attacks on wheat and stimulated fungal growth on nutrient agar. The growth of several strains of Aspergillus and Penicillium in culture were stimulated by 2,4-D. Concentrations of 100 ug/mL enhanced conidium production in Fusarium sp., while concentrations of 1000 ug/mL inhibited conidium production in both Fusarium sp. and Alternaria sp.

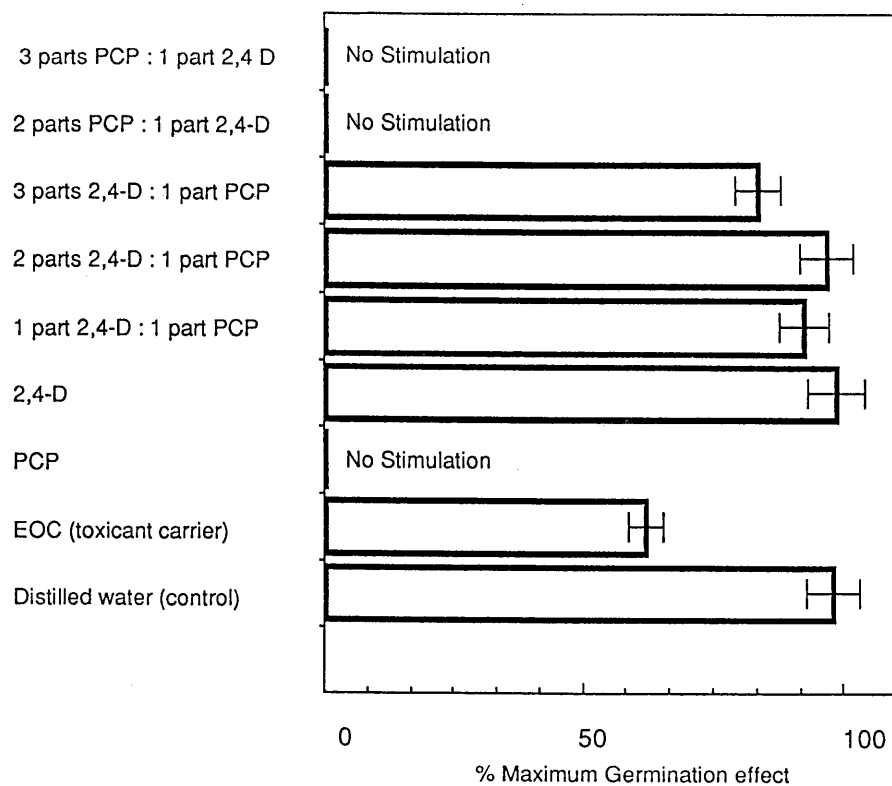


FIGURE 4. Maximum % germination (stimulation) effected by 2,4-D; PCP; or PCP & 2,4-D mixtures. Each bar represents the pooled results of 5 experiments (95% Confidence Limits)

in relatively high proportions binds to and stimulates the remaining unaffected respiratory sites. If these results may be extended to other aquatic fungi e.g., pathogens of rice, the use of 2,4-D to control unwanted aquatic vegetation may actually increase the virulence of some fungal pathogens. Additionally, as single-source contamination is extremely rare, toxicities and relative potential aquatic environmental damage may be increased as a result of concomitant contamination by other pesticides e.g., PCP, that may also be present.

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